# KARYOLOGY AND EFFECTS OF TEMPERATURE AND PHOTOPERIOD ON THE LIFE-HISTORY OF *BANGIA ATROPURPUREA* (ROTH) C. AG. (BANGIALES, RHODOPHYTA) FROM THE MEDITERRANEAN SEA

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ABSTRACT — The effects of temperature, photoperiod and karyology of *Bangia atropurpurea* (Roth) C. Ag. from the Strait of Messina were studied in the field and laboratory. Conchospores were formed and released in short day conditions and at temperature of 15-18° C, with the maximum of production and release at 18° C. Culture data correlate well with field observations on seasonal periodicity of growth and reproduction. Germination and development of monospores and conchospores were not influenced by photoperiod or by light intensity. Temperatures above 25° C were lethal for the all types of spores and gametophytic thalli. The life history in culture corresponds to a typical sexual biphasic cycle with the gametophytic phase showing $n = 3$ chromosomes and the "*Conchocelis*" one $n = 6$. Monospores were produced only by gametophytic plants.

RESUMÉ — La caryologie de *Bangia atropurpurea* (Roth) C. Ag., ainsi que les effets de la température et de la photopériode, ont été étudiés, en laboratoire et dans la nature, sur des échantillons recueillis dans le Détroit de Messine.

Les conchospores se sont différenciées et ont été libérées, en conditions de courte photopériode, à la température de 15-18° C, avec un maximum à 18° C. Les données sur la croissance et la reproduction obtenues en culture concordent avec les observations effectuées dans la nature. La germination et le développement des monospores et des conchospores n'ont pas été influencés par la photopériode ou par l'intensité de la lumière.

Les températures supérieures à 25° C se sont révélées léthales pour les spores et les gamétophytes.

Le cycle biologique de l'algue en culture présente une alternance entre une phase gamétophytique haploïde à n = 3 et une phase « *Conchocelis* » diploïde à 2n = 6. Les monospores sont produites seulement par les gamétophytes.

## INTRODUCTION

The red algal genus *Bangia* is virtually cosmopolitan, being found from subtropical to colder regions of both hemispheres and in both freshwater and marine environments (Laing, 1928; Yabu, 1967; Lee, 1973). Marine populations usually occur high in the intertidal zone in moderately exposed conditions (Tripodi, 1967; Sheath & Cole, 1984).

A sexual life-history, involving the sequential development of two distinct and heteromorphic phases has been described (Drew, 1958; Dixon & Richardson, 1970; Richardson, 1970; Conway & Cole, 1977; Cole & Conway, 1980), and appears to be confirmed on the basis of chromosome counts for the marine species (Yabu, 1967; Magne *in* Cole, 1972; Cole *et al.*,1983). Two further non-sexual life-history types also have been found in certain populations. Firstly, the sequencing of morphological phases proceeds without change in ploidy level (Richardson & Dixon, 1968; Sommerfeld & Nichols, 1970). Secondly, the gametangial phase can repeat by asexual monospores or aplanospores (for a review see West & Hommersand, 1981).

In the closely related genus *Porphyra*, photoperiod, water temperature and light intensity have been demonstrated to control conchospore production and release and seasonal occurrence of the blade phase (Conway & Cole, 1977; Kapraun & Lemus, 1987; Waaland *et al*, 1990; Gargiulo *et al.*, 1994). In *Bangia*, such effects have not been studied in detail (Richardson & Dixon, 1968; Edwards, 1969; Richardson, 1970; Cole, 1972; Sommerfeld & Nichols, 1973; Conway & Cole, 1977) and the factors regulating conchospore release were not studied by these earlier workers. *Bangia atropurpurea* (Roth) C. Ag. is the only species of the genus known from freshwater and marine habitats of Mediterranean region (Honsell & Tripodi, 1964; Tripodi, 1964).

Life-history data for Mediterranean populations, in controlled conditions, are limited to those of Drew (1952, 1958) in which she described different growth stages in marine specimens from Bay of Naples, but chromosome numbers and environmental factors related conchospore production and release and occurrence of the blade phase were not examined. Karyological information for Mediterranean populations are known only for the gametophytic filamentous phase (Gargiulo *et al.*, 1991).

In the present paper we present the results of karyological and culture studies conducted to determine the effects of temperature, photon irradiance and photoperiod on the life history of a Mediterranean marine population of *Bangia atropurpurea* (Roth) C. Ag. from Strait of Messina, Italy.

## MATERIALS AND METHODS

*Bangia atropurpurea* plants were collected from intertidal exposed rocks in the Strait of Messina, Italy (15°40'E, 38°30'N) during the period 1990-1993. Cultures were initiated from zygotospores released from field-collected specimens. The filaments were mechanically isolated under dissecting microscope, gently brushed and washed with sterile seawater to reduce contaminants. Filaments were placed in Petri dishes with sterile seawater. After spore release, the filaments were removed and the spores incubated in a modified von Stosch medium (Guiry & Cunningham, 1984) added with GeO2 5 mg $l^{-1}$. The culture were maintained as a unialgal culture. The culture medium and dishes were changed weekly. Cultures were later transferred to a cross-gradient incubator (Kapraun & Luster, 1980) to test growth and reproduction of conchocelis filaments in a combination of temperature and photoperiod conditions at different photon irradiance levels. Cool-white OSRAM fluorescent lamps produced a photon irradiance at the surface of culture of 5-70 µE mol $m^{-2}$ $s^{-1}$, measured with a LICOR Quantum radiometer model LI 185 A. Photoperiod used were 8L: 16D, 12L: 12D, 16L: 8D and 9L: 15D. Experimental temperatures ranged between 10 and 28° C. About twenty globose tuft of conchocelis filaments were allowed to grow in each dish, and three dishes where used in each experiment. Field observations on local marine

environmental conditions and phenology of *Bangia atropurpurea* were carried out weekly during four years (1990-1993).

Material for chromosome counts were fixed in 3: 1 ethanol: glacial acetic acid (Austin, 1959), and processed as previously described (Kapraun & Gargiulo, 1987).

## RESULTS

Gametophytic filaments appeared in November 1991 and were present until early July 1992. The population consisted of uniseriate (Fig. 1) and three easily distinguishable types of multiseriate filaments: vegetative, male and female (Figs 2-3). Monospores (10-15 × 10-15 µm) were produced in both uniseriate and multiseriate vegetative filaments (Fig. 6). Gametophytic plants formed male and female reproductive structures on separate plants. Male plants produce packets of spermatia in the distal part of the filament. Spermatangia were 15-18 × 10-15 µm and form 32-64 spermatia (2,5-5 × 2,5-5 µm) (Fig. 2). Female plants produced carpogonia (18-22 × 15-18 µm) bearing a single protrichogyne (Fig. 3). Spermatia were observed attached to the protrichogyne with fertilisation canal entering the carpogonium (Fig. 4). Developing zygotosporangia (see Guiry, 1990, p. 363-365) (18-22 × 14-19 µm) gave rise to 4-8 zygotospores (7-12 × 7-12 µm) (Fig. 5).

The presence in the field of the filaments with different spore types or gametes is shown in Table 1. The intertidal sea temperature where field observations were carried out ranged between 15° C in February and 25° C in August. The pattern of average sea temperatures as well as hours of light the year round are shown in Fig. 7.

| | Spermatangia | Carpogonia | Zygotospores | Monospores |
|---|---|---|---|---|
| Nov. | - | - | - | + |
| Dec. | + | + | + | + |
| Jan. | + | + | + | + |
| Feb. | + | + | + | + |
| Mar. | + | + | + | + |
| Apr. | + | + | + | + |
| May | + | + | + | + |
| Jun | + | + | + | + |
| Jul. | + | - | - | + |
| Aug. | - | - | - | - |
| Sep. | - | - | - | - |
| Oct. | - | - | - | - |
| Nov. | - | - | - | + |

Table 1. Presence in the field of different spores types or gametes of *Bangia atropurpurea* during the period 1991-1993.

Zygotospores germinated in culture, 4-10 days after release, in a unipolar manner resulting in loose tufts of branched filaments, the conchocelis stage (Fig. 12). Zygotospores were released and germinated in all the conditions of photon irradiance, temperature and photoperiod tested (Tab. 2); gametophytic thalli and released spores at temperatures higher than 25° C soon died.

| Photoperiod (h light, h dark) | Temperature (°C) | Zygotospore release | Zygotospore germination |
|---|---|---|---|
| 8 : 16 | 10 | - | + |
| | 15 | + | + |
| | 18 | + | + |
| | 22 | + | + |
| | 25 | - | - |
| | 28 | - | - |
| 12 : 12 | 10 | + | + |
| | 15 | + | + |
| | 18 | + | + |
| | 22 | + | + |
| | 25 | - | - |
| | 28 | - | - |
| 16 : 8 | 10 | + | + |
| | 15 | + | + |
| | 18 | + | + |
| | 22 | + | + |
| | 25 | - | - |
| | 28 | - | - |

Table 2. Effect of photoperiod and temperature on the zygotospore release and germination of *Bangia atropurpurea* at 50 µE $m^{-2}$ $s^{-1}$.

Conchocelis filaments transferred at temperatures between 15 and 22° C formed conchosporangia under daylengths of 8L: 16D (Fig. 13). Maximal conchosporangial production was observed at 18° C with 60 % of plants differentiating spores. Only few plants (± 5 %) formed conchosporangia at 15° C. Filaments of conchocelis stage grew under short-day photoperiod at temperatures lower than 15° C never produced conchosporangia. A few spores were produced (10 % of plants) at temperatures between 18 and 22° C under 12L: 12D photoregime. Conchocelis grown under long day conditions (16L: 8D) never differentiated conchosporangia at any temperature and photon irradiance tested. The effects of photoperiod and temperature on the differentiation of conchosporangia on the conchocelis stage are shown in Table 3.

The conchocelis filaments released conchospores only under short-day conditions at 15-22° C (40-50 % of plants) with a maximum of release under 9L: 15D photoperiod at 18° C (100 % of plants). A small numbers of plants (10 %) released spores at 18-22° C, 12L: 12D. The effects of photoregime and temperature on the release of spores from the conchocelis phase are summarised in Table 4.

Conchospores germinated, in all the tested conditions, in a bipolar manner forming new gametophytic plants (Figs 8-11).

Production of monospores from gametophytic thalli was observed both in the field or in culture. These germinated in a bipolar way resulting in a new gametophytic plants (Figs 8-11). Germination was obtained at all the temperatures between 10 and 25° C and at all the photon irradiances tested. At temperatures higher than 25° C, the spores failed to germinate and soon died (Tab. 5).

Formation of monospores on the conchocelis phase was not observed; however, a frequent fragmentation of the filamentous tufts was observed, increasing the number of thalli in culture.

Haploid chromosome numbers $n = 3$ and diploid $2n = 6$ were observed in spermatangia and vegetative cells of conchocelis phase respectively (Figs 14-15).

| Photoperiod (h light: h dark) | Temperature (°C) | % of conchocelis thalli producing sporangia |
|---|---|---|
| 8 : 16 | 10 | Filaments remain vegetative |
| | 15 | 5 % conchosporangia formed |
| | 18 | 60 % conchosporangia formed |
| | 22 | 50 % conchosporangia formed |
| | 25 | - |
| | 28 | - |
| 12 : 12 | 10 | Filaments remain vegetative |
| | 15 | Filaments remain vegetative |
| | 18 | 10 % conchosporangia formed |
| | 22 | 10 % conchosporangia formed |
| | 25 | - |
| | 28 | - |
| 16 : 8 | 10 | Filaments remain vegetative |
| | 15 | Filaments remain vegetative |
| | 18 | Filaments remain vegetative |
| | 22 | Filaments remain vegetative |
| | 25 | - |
| | 28 | - |

Table 3. Effect of photoperiod and temperature on the concospore formation of *Bangia atropurpurea* at 50 μE $m^{-2}$ $s^{-1}$.

| Photoperiod (h light: h dark) | Temperature (°C) | % of conchocelis thalli releasing spores |
|---|---|---|
| 8 : 16 | 10 | No release |
| | 15 | 10 % conchospore released |
| | 18 | 60 % conchospore released |
| | 22 | 50 % conchospore released |
| 9 : 15 | 10 | No release |
| | 15 | No release |
| | 18 | 100 % conchospore released |
| | 22 | No release |
| 12 : 12 | 10 | No release |
| | 15 | No release |
| | 18 | 10 % conchospore released |
| | 22 | 10 % conchospore released |
| 16 : 8 | 10 | No release |
| | 15 | No release |
| | 18 | No release |
| | 22 | No release |

Table 4. Effect of photoperiod and temperature on the concospore release of *Bangia atropurpurea* at 50 μE $m^{-2}$ $s^{-1}$.

| Photoperiod (h light: h dark) | Temperature (°C) | Monospore release | Monospore germination |
|---|---|---|---|
| 8 : 16 | 10 | + | + |
| | 15 | + | + |
| | 18 | + | + |
| | 22 | + | + |
| | 25 | - | - |
| | 28 | - | - |
| 12 : 12 | 10 | + | + |
| | 15 | + | + |
| | 18 | + | + |
| | 22 | + | + |
| | 25 | - | - |
| | 28 | - | - |
| 16 : 8 | 10 | + | + |
| | 15 | + | + |
| | 18 | + | + |
| | 22 | + | + |
| | 25 | - | - |
| | 28 | - | - |

Table 5. Effect of photoperiod and temperature on the monospore release and germination of *Bangia atropurpurea* at 50 μE $m^{-2}$ $s^{-1}$.

## DISCUSSION

*Bangia atropurpurea* in the studies isolates demonstrated a heteromorphic life-cycle involving an alternation between a macroscopic phase and a microscopic filamentous conchocelis phase. Chromosome counts of $n = 3$ and $2n = 6$ in the gametophytic and sporophytic phases, respectively, indicate a presence of sexuality and meiosis in these isolates; this conclusion is also supported by the observation of spermatia attached to protrichogyne and the development of a fertilization canal entering the carpogonium. *Bangia atropurpurea* populations from North Atlantic and Pacific Oceans are reported to have similar life histories, however some of these showed 4 and 8 chromosomes in the haploid and diploid phases respectively (Yabu, 1967; Magne *in* Cole, 1972; Cole *et al.*, 1983). By contrast, freshwater and certain marine populations are reported to reproduce only asexually (Nichols & Veith, 1978; Sheath & Cole, 1980).

Culture data suggest that the conchocelis phase should grow in the field all year round. By contrast, as reported for most species of the closely related genus *Porphyra* (Waaland *et. al.*, 1987; Waaland *et al.*,1990; Gargiulo *et al.*, 1994), conchosporangial maturation and conchospore release in *Bangia* isolates from Sicily are under photoperiodic and temperature control, occurring only during short days at temperature between 15 and 22° C with a maximum of production and release at 18° C. These data correlate well with the conditions observed at the time when first stages of gametophytic thalli are recorded in nature.

Under culture conditions either gametophytic and sporophytic filaments either all type of spores are not able to survive at an temperature higher than 25° C, corresponding to that observed in the field when the macroscopic phase begins to disappear. On the other hand, germination and development of monospore and

conchospore are not influenced by photoperiod and photon irradiance levels as shown also by Edwards (1969) and Cole (1972), emphasizing true potential of these structures to assure the presence of macroscopic thalli throughout the year. According to Cole (1972), zygotospore release and germination in our isolates are not influenced by photoperiod; however, Richardson (1970) and Richardson & Dixon (1968) observed in populations from Washington and British Columbia a unipolar or bipolar germination of zygotospores when grown under different photoregimes.

In conclusion, temperature and photoperiod appear to be critical ecological factors acting as a seasonal trigger involved in the regulation of the alternation of macroscopic and conchocelis phases of *Bangia* populations in nature. Temperature seems to be the most important factor, acting directly or indirectly on the metabolism of gametophytic plants.

Further studies, particularly of populations from other geographical regions and from freshwater, are necessary for an understanding of the biology, phenology and geographical distribution of *Bangia*.

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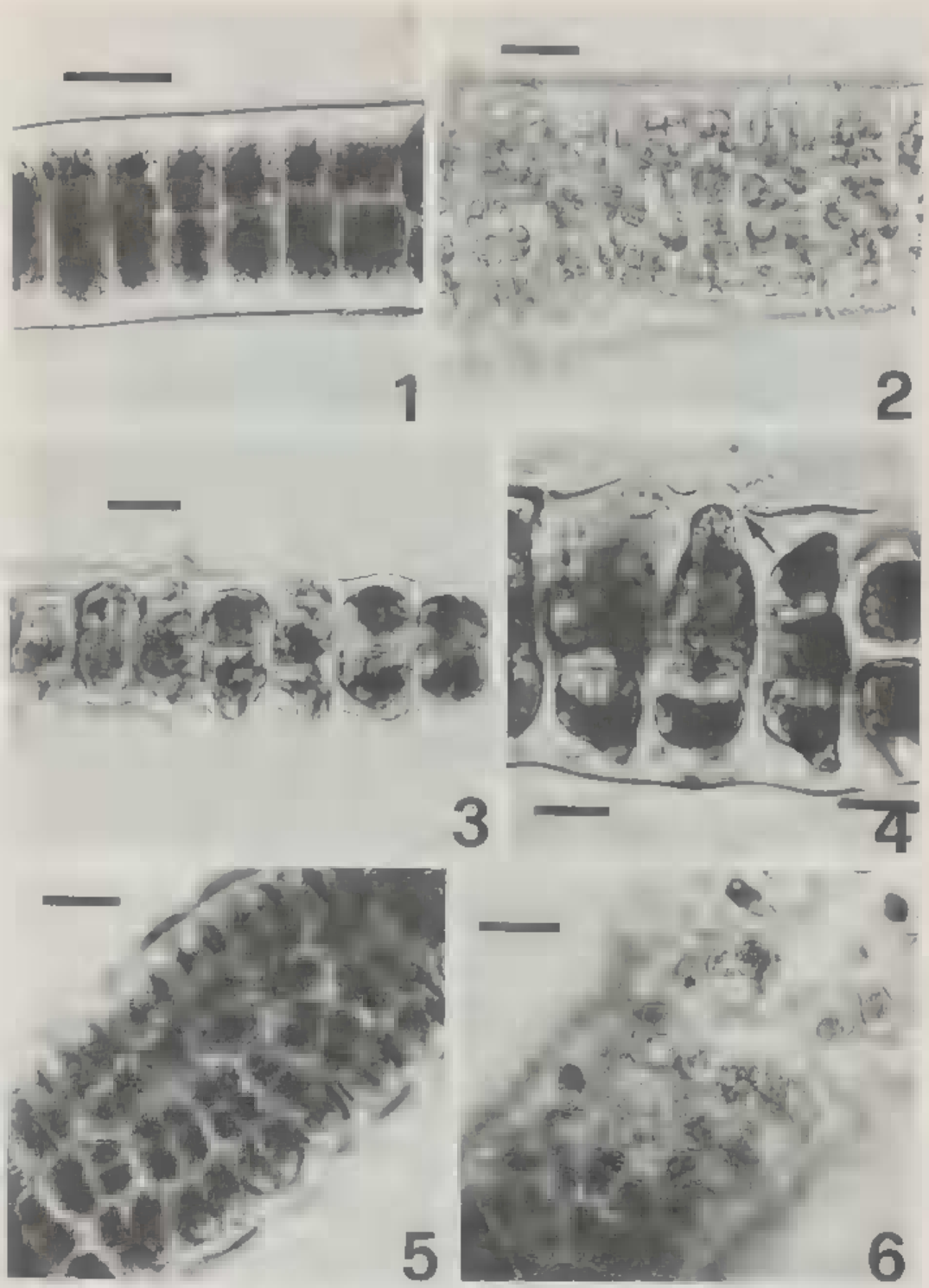



Figs 1-6. *Bangia atropurpurea* (Roth) C. Ag.-Fig. 1. Initially uniseriate filaments becoming multiseriate by successive divisions of the cells. Scale = 20 µm. Fig. 2. Male gametophytic plant producing packets of colorless spermatia. Scale = 20 µm. Fig. 3. Female gametophytic plant with carpogonia bearing a single trichogyne. Scale = 20 µm. Fig. 4. Carpogonium with spermatium attached to the trichogyne. Note the fertilization canal (→) entering the carpogonium Scale = 10 µm. Fig. 5. Zygotosporangia. Scale bar = 20 µm. Fig. 6. Monospores. Scale bar = 20 µm.

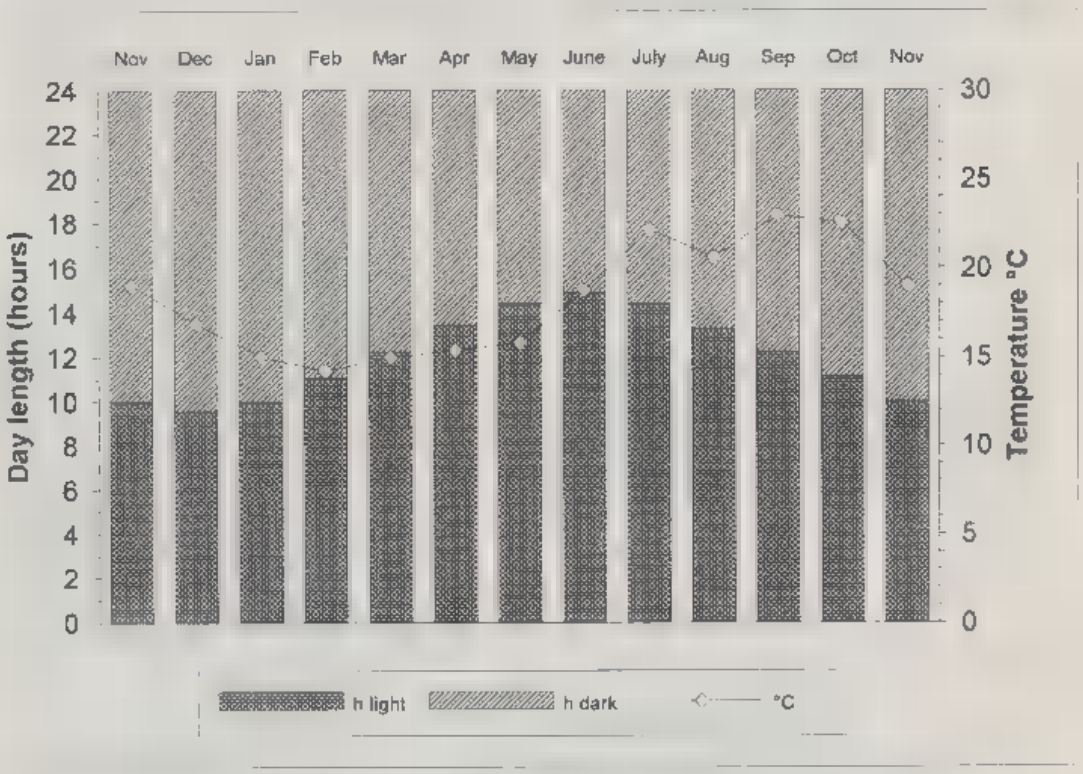
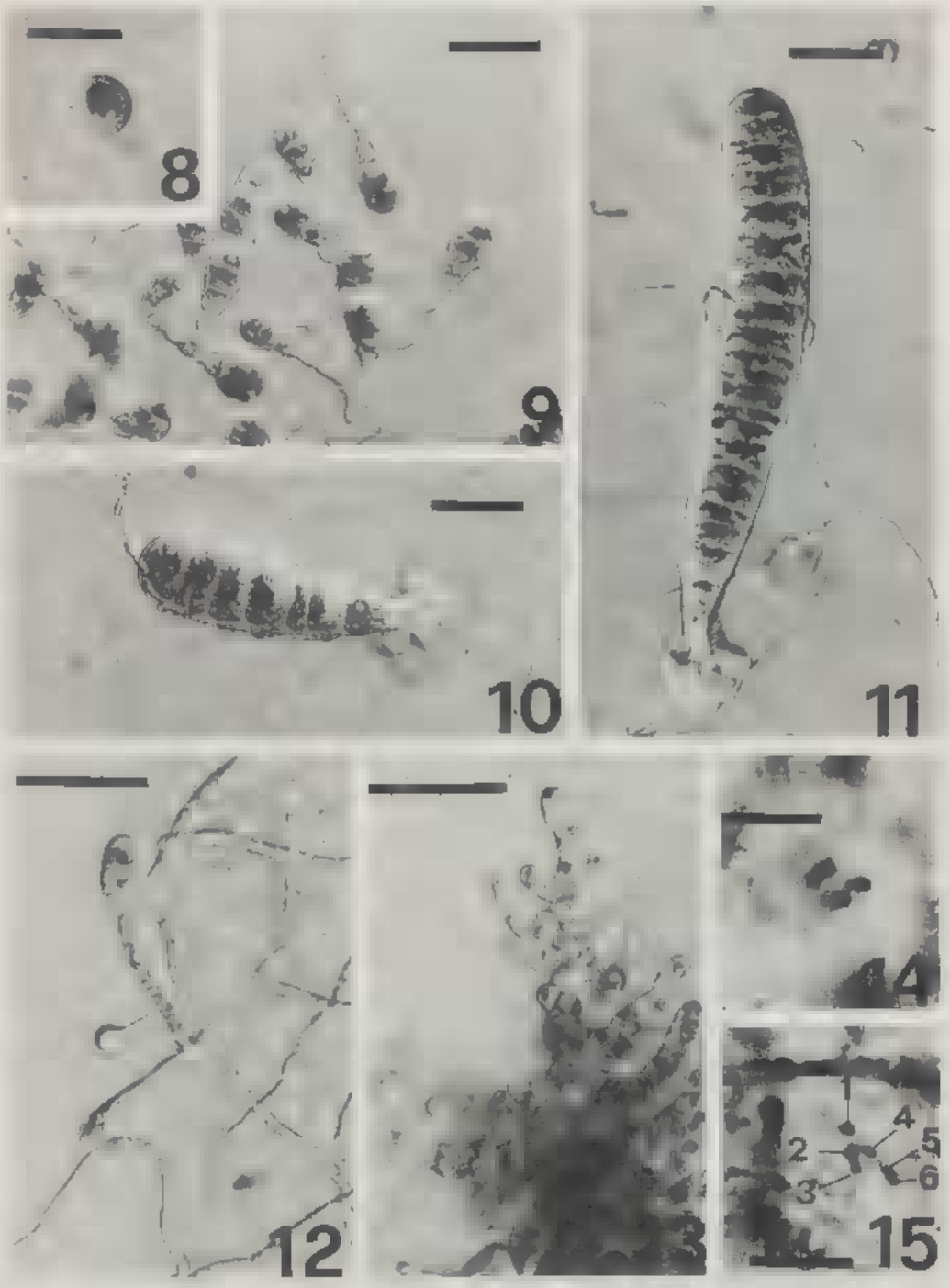



Fig. 7. *Bangia atropurpurea* (Roth) C. Ag.-Average sea temperatures and hour of light year round at the collecting site in the Strait of Messina (15°40′ E, 38°30′ N) during the period.




Figs 8-15. *Bangia atropurpurea* (Roth) C. Ag.-Figs 8-11. Bipolar model of germination resulting in a new gametophytic thallus. Scale bars = 20 µm. Fig. 12. Conchocelis stage. Note first steps of conchosporangia formation. Scale bar = 40 µm. Fig. 14. Spermatangium, late prophase: $n$ = 3. Scale bar = 5 µm. Fig. 15. Conchocelis vegetative cell: $n$ = 6. Scale bar = 5 µm.